Site-Specific Protein Transamination Using *N*-Methylpyridinium-4-carboxaldehyde

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Supporting Information

General Procedures. Unless otherwise noted, the chemicals and solvents used were of analytical grade and were used as received from commercial sources. Water (dd-H_2O) used as reaction solvent and in biological procedures was deionized using a Barnstead NANOpure purification system (ThermoFisher, Waltham, MA). *N*-methylpyridinium-4-carboxaldehyde benzenesulfonate salt (Rapoport's salt, RS) was obtained from Alfa Aesar. Pyridoxal 5'-phosphate monohydrate was obtained from Aldrich. All Fmoc-protected amino acids were obtained from Novabiochem (EMD, Germany). Tentagel S OH resin was obtained from Advanced ChemTech (Louisville, KY). Wild-type anti-HER2 human IgG1 monoclonal antibodies were obtained from Eureka Therapeutics, Inc (Emeryville, CA). Wild-type anti-hTNFα human IgG1 monoclonal antibodies were obtained from Invivogen (San Diego, CA). Goat anti-human IgG (Fcγ specific) PerCP-conjugated $F(ab')_2$ fragments were purchased from Jackson ImmunoResearch Laboratories, Inc (West Grove, PA). Centrifugations were conducted with an Eppendorf Mini Spin Plus (Eppendorf, Hauppauge, NY).

Solid-Phase Peptide Synthesis. Peptides were synthesized using standard conditions for Fmoc-based chemistry. The side chain protecting groups used were: Asn(Trt), Asp(tBU), Arg(Pbf), Cys(Trt), Gln(Trt), Glu(tBu), His(Trt), Lys(Boc), Ser(tBu), Thr(tBu), Trp(Boc), Tyr(tBu). For the first step, the C-terminal amino acid (10 equiv) was preactivated with 5 equivalents of diisopropylcarbodiimide (DIC), then coupled to the Tentagel S OH resin with 0.1 equivalents of *N*,*N*-dimethylaminopyridine (DMAP) as an additive. Deprotection of the Fmoc groups was accomplished by incubation with a 20% v/v piperidine/*N*,*N*-dimethylformamide (DMF) solution for 20 min. Subsequent coupling reactions were carried out using 10 equivalents of amino acid with 10 equivalents of 2-(6-chloro-1-*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) and 20 equivalents of *N*,*N*-diisopropylethylamine (DIPEA) in DMF for 10 minutes. Side-chain deprotection was achieved using a 1-2 h incubation with a 95:2.5:2.5 ratio of trifluoroacetic acid (TFA) to H_2O to triisopropylsilane (TIPS), followed by equilibration in 50 mM phosphate buffer (pH 6.5) using three 5 min exposures.

Split-Pool Library Synthesis with Partial Truncation. The construction of the one-bead-one-sequence combinatorial peptide library with a built-in truncation ladder followed the previously reported technique.[1] The synthesis began with a 5-residue base sequence (WSNAG) prepared on 500 mg of Tentagel S OH resin. Partial capping was performed using 0.15 equivalents of bromobenzoic acid, 0.1 equivalents of 2-(6-chloro-1-*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) and 1 equivalent of *N*,*N*-diisopropylethylamine (DIPEA). The resin was then split into 20 different reaction vials, and each portion was exposed to peptide coupling conditions (10 equivalents of amino acid with 10 equivalents of HCTU and 20 equivalents of DIPEA in DMF for 10 minutes) using one of the twenty amino acids. After coupling and rinsing, the resins were then recombined, with the exception of the

Gln and Leu samples, which were kept separate. After Fmoc deprotection, the next capping step was performed. Bromobenzoic acid was attached to the combined resin portion and methylbromobenzoic acid was used to cap the Gln and Leu samples. The Gln and Leu samples were mixed with the rest of the resin before splitting the combined library into separate vials for the next coupling reaction.

General Procedure for Library Sequencing. Individual beads identified in the library were incubated with 10 μL of 100 mM NaOH solution to cleave the peptide from the resin. The solution was desalted using Ziptips with 0.2 μL of C18 resin (Millipore, Billerica, MA). The peptide was eluted from the Ziptip with 2 μL of matrix solution (described below) directly onto a MALDI sample plate (Applied Biosystems, Foster City, CA). MALDI-TOF analysis was performed on a Voyager-DE instrument (Applied Biosystems) using reflector mode, and all spectra were analyzed using Data Explorer software. The truncation ladder peaks were identified from their bromine isotope pattern and the mass differences between these peaks were used to determine the sequence.[1] The matrix solution was a saturated α-Cyano-4-hydroxycinnamic acid solution in 50% acetonitrile, 50% water, 0.1% TFA.

Construction of Light Chain Anti-HER2 Human IgG1 Expression Plasmids. To clone a plasmid for the expression of the anti-HER2 human IgG1 light chain, the sequence for the variable domain of the light chain (V_L) was obtained from the literature,[2] assembled into a gene, then cloned into a plasmid containing the light chain constant region. Gene2Oligo was used to generate the following set of oligonucleotides for gene assembly from the V_L sequence. An IL2 signaling sequence was also included in the N-terminal region: (The bases in lower case were added by the Gene2Oligo program and did not belong to the input sequence):

R0 ACCTTTTTTTacattgaagtgcag
F0 ctgcacttcaatgtAAAAAAAGGTCACCATGTACAGGATGCA
R24 GCAATGCAAGACAGGAGTTGCATCCTGTACATGGTG
F42 ACTCCTGTCTTGCATTGCACTAAGTCTTGCACTTGTCA
R60 TCAGTCTTAGCCGAATTCGTGACAAGTGCAAGACTTAGT
F80 CGAATTCGGCTAAGACTGACATCCAAATGACTCAGAGCC
R99 GCGCTCAGGGAACTGGGGCTCTGAGTCATTTGGATG
F119 CCAGTTCCCTGAGCGCTTCCGTAGGGGACAGG
R135 GCCCGACATGTTATTGTCACCCTGTCCCCTACGGAA
F151 GTGACAATAACATGTCGGGCTAGCCAGGATGTCAATACAG
R171 CTGGTACCAAGCGACAGCTGTATTGACATCCTGGCTA
F191 CTGTCGCTTGGTACCAGCAAAAGCCCGGAAAGGC
R208 GCTGTATATAAGAAGCTTTGGCGCCTTTCCGGGCTTTTG
F225 GCCAAAGCTTCTTATATACAGCGCCAGTTTCCTCTATTCTGG
R247 GAACCTGCTCGGCACGCCAGAATAGAGGAAACTGGC
F267 CGTGCCGAGCAGGTTCTCTGGATCTCGGTCCG
R283 TCAGTGTGAAATCGGTCCCGGACCGAGATCCAGA
F299 GGACCGATTTCACACTGACCATTAGTTCTCTGCAGCC
R317 TAGTATGTTGCAAAGTCCTCTGGCTGCAGAGAACTAATGG
F336 AGAGGACTTTGCAACATACTACTGCCAGCAGCACTAT
R357 AGGTTGGGGGTGTGGTATAGTGCTGCTGGCAG
F373 ACCACACCCCCAACCTTTGGTCAGGGCACGAA
R389 CGTACGCTTGATTTCCACCTTCGTGCCCTGACCAA
F405 GGTGGAAATCAAGCGTACGAAAAAAAcccccaactttgt
F424 acaaagttgggggTTTTTTT

An additional GCTAAAACT was added to the 5' end according to a published procedure[3] in order

to create a three residue N-terminal extension (AKT, for use in other work and later mutated to EES for this work). The resulting V_L gene was inserted into a vector at BsiWI and BstEII restriction sites using standard cloning techniques. The vector used, pFUSE2-CLIg-hk from Invivogen (San Diego, CA), already contained the constant region of the kappa light chain (Figure S8) A Quikchange site-directed mutagenesis kit (Stratagene, La Jolla, CA) was used to generate the desired N-terminal mutant (EES). Incorporation of these mutations was verified by sequencing.

Construction of Heavy Chain Anti-HER2 Human IgG1 Expression Plasmids. A plasmid for the expression of the anti-HER2 heavy chain was cloned in a similar fashion to that of light chain. In brief, the variable and constant region 1 of heavy chain (V_H and C_H1) was constructed from the following set of oligonucleotides with additional bases (CTCCAAACA) at the 5' end (corresponding to three N-terminal residues, LQT, for use in other work and later mutated to EES for this work).

R0 TTTTTTTcttagctgctttga
F0 tcaaagcagctaagAAAAAAAGAATTCGCTCCAAACAG
R21 CGACGAGTTGGACTTCTGTTTGGAGCGAATTC
F38 AAGTCCAACTCGTCGAAAGCGGAGGTGGC
R53 CCAGGCTGAACCAGGCCACCTCCGCTTT
F67 CTGGTTCAGCCTGGCGGAAGCCTGCGC
R81 GCAGCACAGCTCAAGCGCAGGCTTCCG
F94 TTGAGCTGTGCTGCCTCCGGATTTAATATCAAAGA
R108 CGAACCCAGTGTATATAAGTATCTTTGATATTAAATCCGGAG
F129 TACTTATATACACTGGGTTCGCCAGGCTCCTGGA
R150 CCACTCCAGACCCTTTCCAGGAGCCTGG
F163 AAGGGTCTGGAGTGGGTGGCGAGAATCTACC
R178 GGGTATAACCATTGGTTGGGTAGATTCTCGCCAC
F194 CAACCAATGGTTATACCCGCTATGCAGACAGCG
R212 GTAAACCGCCCTTTCACGCTGTCTGCATAGC
F227 TGAAAGGGCGGTTTACAATTAGTGCCGACACA
R243 GGTAAGCGGTATTTTTAGATGTGTCGGCACTAATT
F259 TCTAAAAATACCGCTTACCTCCAGATGAACTCTCTG
R278 TGTCCTCGGCCCTCAGAGAGTTCATCTGGA
F295 AGGGCCGAGGACACGGCTGTGTATTATTGC
R308 CACCCCACCGGCTGCAATAATACACAGCCG
F325 AGCCGGTGGGGTGGAGACGGATTCTATGCT
R338 TGACCCCAATAGTCCATAGCATAGAATCCGTCTC
F355 ATGGACTATTGGGGTCAGGGCACTCTCGTCA
R372 TGGCACTGCTTACAGTGACGAGAGTGCCC
F386 CTGTAAGCAGTGCCAGCACAAAGGGGCC
R401 CAAGGGGAAAGACACTAGGCCCCTTTGTGC
F414 TAGTGTCTTTCCCCTTGCTCCATCTAGCAAATCTAC
R431 GGTGCCCCCGCTGGTAGATTTGCTAGATGGAG
F450 CAGCGGGGGCACCGCCGCCCTGGGAT
R463 GTCCTTGACCAGGCATCCCAGGGCGGC
F476 GCCTGGTCAAGGACTATTTTCCTGAGCCAGT
R490 TCCAGGACACGGTGACTGGCTCAGGAAAATA
F507 CACCGTGTCCTGGAATAGTGGCGCCTTGA
R521 TGTGTGAACACCAGAAGTCAAGGCGCCACTAT
F536 CTTCTGGTGTTCACACATTTCCCGCCGTCC
R553 CAGCCCACTAGATTGAAGGACGGCGGGAAA

F566 TTCAATCTAGTGGGCTGTACTCTCTCTCCAGTGT
R583 TGGGTACCGTCACCACACTGGAGAGAGAGTA
F600 GGTGACGGTACCCAGTTCAAGCTTGGGCA
R614 TGCAGATATAGGTCTGTGTGCCCAAGCTTGAAC
F629 CACAGACCTATATCTGCAATGTGAACCACAAGCC
R647 CCACCTTTGTATTGCTGGGCTTGTGGTTCACAT
F663 CAGCAATACAAAGGTGGACAAAAAAGTCGAGCCT
R680 TGTCACAGCTCTTTGGAGGCTCGACTTTTTTGT
F697 CCAAAGAGCTGTGACAAAACTCACACATGCCC
R713 TACCTGGGCACGGTGGGCATGTGTGAGTTT
F729 ACCGTGCCCAGGTAAGCCAGCCCAGGC
R743 ccccattgactTTTTTTTAGGCCTGGGCTGGCT
R756 CTAAAAAAAagtcaatgggg

The BglII site was introduced using PCR with forward primer F0 and a reverse primer containing a BglII restriction site (sequences shown below).

Forward: tcaaagcagctaagAAAAAAAGAATTCGCTCCAAACAG
Reverse: ttttttAGATCTCTTTGGAGGCTCGACTTTTTTGT

The gene encoding V_H and C_H1 was inserted into a vector comprising the crystallizable fragment (F_c) domain (i.e. C_H2 and C_H3 domains) of human IgG1 heavy chain (pinfuse-higG1-fc2 from Invivogen) at the EcoRI and BglII restriction sites (Figure S8). A Quikchange site-directed mutagenesis kit was used to generate the desired N-terminal mutant (EES). Incorporation of these mutations was verified by sequencing.

General procedure for expression and purification of mutant antibodies. The plasmids for the light and heavy chains of the anti-HER2 antibody were transiently co-transfected into human embryonic kidney 293T cells in a 3:2 ratio using lipofectamine 2000 (Invitrogen, Grand Island, NY) in Opti-MEM medium following the protocol from Invitrogen. The cells were incubated at 37 ˚C in 5% CO_2. After two days, the media was collected and the secreted antibodies were purified using protein G affinity chromatography, according to the procedure from the manufacturer (Pierce, Rockford, IL). The media was replaced and cultures were grown for an additional 3 days, after which the additional antibodies were harvested and purified as above. Purified protein was buffer exchanged into PBS using Amicon Ultra 4 mL 10,000 MWCO (Millipore) centrifugal ultrafiltration membranes. Purity was evaluated by SDS-PAGE with Coomassie staining.

General Procedure for Antibody Disulfide Reduction and Cysteine Capping for Mass Spectrometry Analysis. To prepare the antibody mutants for mass spectrometry analysis, first the oligosaccharides were removed via treatment with N-Glycosidase F (PNGase F) following the protocol from the manufacturer (New England Biolabs, Ipswich, MA). Briefly, a buffer exchange into PBS was performed on the antibody samples. In a 1.5 mL Eppendorf tube, the protein was mixed with 10 µL of G7 reaction buffer, 4 µL PNGase, and additional PBS to a total volume of 100 µL. The mixture was then incubated at 37 ºC overnight. Immediately following treatment with PNGase, buffer exchange was performed into 100 mM Tris buffer, pH 8. Dithiothrietol (DTT) and ethylenediaminetetraacetic acid (EDTA) were then added to a final concentration of 10 mM each, and the reaction was incubated at room temperature for 20 min. After the reduction, iodoacetamide was added to a final concentration of 50 mM and the mixture was incubated at 37 ºC for 30 min. The samples were then subjected to buffer exchange into 10 mM Tris buffer pH 8 for mass spectrometry analysis.

Liquid Chromatography and Mass Spectrometry Materials. Acetonitrile (Fisher Optima grade, 99.9%), formic acid (Pierce, 1 mL ampules, 99+%), and water purified to a resistivity of 18.2 MΩ·cm (at 25 °C) using a Milli-Q Gradient ultrapure water purification system (Millipore, Billerica, MA) were used to prepare mobile phase solvents for liquid chromatography-mass spectrometry (LC-MS).

LC-MS and MS/MS Analysis of Synthetic Peptide Bioconjugates. Peptide bioconjugates were analyzed using a nanoAcquity ultraperformance liquid chromatograph (UPLC; Waters, Milford, MA) that was connected in-line with a quadrupole time-of-flight mass spectrometer (Q-Tof Premier, Waters).

The UPLC was equipped with C18 trapping (5 μm particles, 20 mm × 180 μm) and analytical (1.7 μm particles, 100 mm × 100 μm) columns and a 10 μL sample loop. Solvent A was 99.9% water/0.1% formic acid and solvent B was 99.9% acetonitrile/0.1% formic acid (v/v). Sample solutions contained in 0.3 mL polypropylene snap-top vials sealed with septa caps were loaded into the nanoAcquity autosampler compartment prior to analysis. Following sample injection (1 μL, partial loop), trapping was performed for 2 min with 100% A at a flow rate of 15 μL/min. The injection needle was washed with 500 μL of A and 200 μL of B after injection to avoid cross-contamination between samples. The elution program consisted of a linear gradient from 12% to 75% B over 15 min, a linear gradient to 95% B over 0.33 min, isocratic conditions at 95% B for 2.67 min, a linear gradient to 1% B over 0.33 min, and isocratic conditions at 1% B for 10.67 min, at a flow rate of 500 nL/min. The analytical column and sample compartment were maintained at 35 ºC and 8 ºC, respectively.

The column exit was connected to a Universal Nanoflow Sprayer nanoelectrospray ionization (nanoESI) emitter mounted in the nanoflow ion source of the Q-TOF mass spectrometer. The nanoESI source parameters were as follows: nanoESI capillary voltage 2.4 kV, nebulizing gas (nitrogen) pressure 0.15 mbar, sample cone voltage 35 V, extraction cone and ion guide voltages both 4 V, and source block temperature 80 ºC. Cone gas was not used. The collision cell contained argon gas at a pressure of 8×10^{-3} mbar. The Tof analyzer was operated in "V" mode and routinely achieved a mass resolving power of 1.0×10^4 (measured at $m/z = 498$, full width at half maximum peak height), which was sufficient to resolve the isotopic distributions of singly and multiply charged peptide ions. Thus, a peptide ion's mass and charge could be determined independently (i.e., the ion charge was determined from the reciprocal of the spacing between adjacent isotope peaks in the m/z spectrum). External mass calibration of the Tof analyzer was performed immediately prior to analysis using a sodium formate solution. Mass spectra were acquired in the positive ion mode over the range m/z = 300-1500, in continuum data format, using a 0.95 s scan integration and a 0.05 s interscan delay. In the data-dependent mode, up to three precursor ions exceeding an intensity threshold of 30 counts/second (cps) were selected from each survey scan for tandem mass spectrometry (MS/MS) analysis. MS/MS spectra were acquired over the range m/z = 100-1500 using a 0.45 s scan integration, a 0.05 s interscan delay, and a collision energy of 30 eV. Ions were fragmented to achieve a minimum total ion current (TIC) of 250,000 cps in the cumulative MS/MS spectrum for a maximum of 10 s. An include list was used to select precursor ions of interest for MS/MS preferentially. To avoid the occurrence of redundant MS/MS measurements, real-time dynamic exclusion was used to preclude re-selection of previously analyzed precursor ions over an exclusion width of ±0.2 m/z unit for a period of 120 s. Mass spectra and MS/MS spectra were processed using MassLynx software (version 4.1, Waters).

LC-MS Analysis of Reduced Antibody Bioconjugates.

Electrospray ionization mass spectrometry (ESI-MS) of peptides was performed using an Agilent 1260 series liquid chromatograph outfitted with an Agilent 6224 Time-of-Flight (TOF) LC-MS system (Santa Clara, CA).

The LC was equipped with a Poroshell 300SB-C18 (5 μm particles, 1.0 mm × 75 mm, Agilent, Santa Clara, CA) analytical column. Solvent A was 99.9% water/0.1% formic acid and solvent B was 99.9% acetonitrile/0.1% formic acid (v/v). Sample solutions contained in 0.3 mL polypropylene snap-top vials

sealed with septa caps were loaded into the Agilent 1260 autosampler compartment prior to analysis. For each sample, approximately 5 to 10 picomoles of analyte was injected onto the column. Following sample injection, a 20-100% B elution gradient was run at a flow rate of 0.55 mL/min for 7 min. Data was collected and analyzed using Agilent MassHunter Qualitative Analysis B.05.00.

Flow Cytometry Analysis of Antibody Bioconjugates.

MCF7 clone 18 (MCF7cl18), a human breast cancer cell line overexpressing HER2, cells were obtained from the Preclinical Therapeutics Core Facility, UCSF, and grown in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) at 37 °C in 5% CO_2. Jurkat cells (negative control) were obtained from ATCC and grown in RPMI containing 10% FBS. For the binding experiment, MCF7 clone 18 cells were first trypsinized at 37 °C for 5 min, followed by the addition of complete media (DMEM + 10% FBS) to stop trypsinization. Both MCF7 clone 18 and Jurkat cells were then pelleted and resuspended in binding buffer (Dulbecco's phosphate buffered saline (DPBS) containing 1% FBS) to the density of $1x10^6$ cells/mL. Aliquots of 100 µL containing $1x10^6$ cells/mL of cells were incubated with 15 and 25 nM of the unmodified or modified antibodies for 45 min on ice. The cells were then washed twice with 150 µL and resuspended in 100 µL of binding buffer containing 1:1000 dil. goat anti-human IgG, Fcγ fragment specific PerCP-conjugated. The cells were incubated for 30 min on ice in the dark, then washed twice with 150 µL, and resuspended in 200 µL of binding buffer. The cells were analyzed by flow cytometry (FACSCalibur flow cytometer, BD Biosciences) to determine the amount of AlexaFluor 488 and PerCP fluorescence. For each sample, 10,000 cells were counted.

Supplemental References:

1. Witus, L. S.; Moore, T.; Thuronyi, B. W.; Esser-Kahn, A. P.; Scheck, R. A.; Iavarone, A. T.; Francis, M. B. *J. Am. Chem. Soc.* **2010**, *132*, 16812–16817.
2. Cho, H.-S.; Mason, K.; Ramyar, K. X.; Stanley, A. M.; Gabelli, S. B.; Denney, D. W.; Leahy, D. J. *Nature* **2003**, *421*, 756–760.
3. Rouillard, J.-M.; Lee, W.; Truan, G.; Gao, X.; Zhou, X.; Gulari, E. *Nucl. Acids Res.* **2004**, *32*, 176–180.
4. Papayannopoulos, I.A. *Mass Spectrom Rev* **1995**, *14*, 49–73.

Supporting Figures:

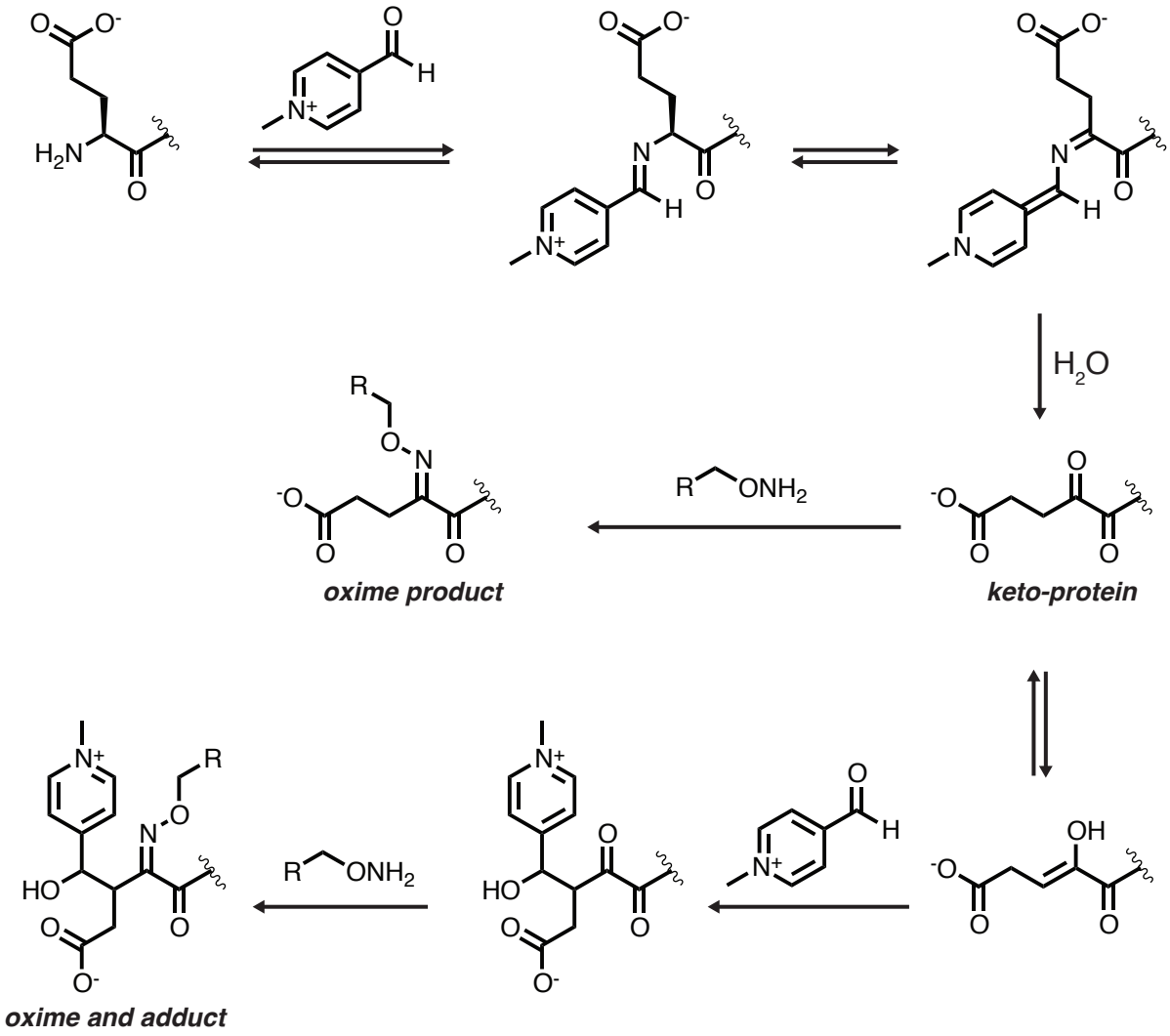


Figure S1. Proposed mechanism of RS-mediated transamination. The N-terminus of the protein first forms a Schiff base with the aldehyde. Alleviation of the charge on the pyridinium nitrogen helps drive the next step, which is deprotonation of the N-terminal α-hydrogen. Hydrolysis leads to the transaminated keto-protein species, which is then able to form the desired oxime product upon incubation with an alkoxyamine reagent of choice. As a mass corresponding to the addition of RS is observed, a likely reaction pathway for this is an aldol reaction with another equivalent of RS. This structure leads to the oxime and adduct species after reaction with the alkoxyamine.

library:	*beads selected:*	*sequences identified:*
entire library	red	EEQ, EED, EEI, EEM, ENE, EDD, EDW (2x), EDN, EDH, ETE, DEE, FED, FDM, FEE, WEE, WDD, YEE
E-terminal subset of library (EXX)	red	EES (3x), EEE, EEW, EET, EEN, EEY, EEA, EDE (3x), EDH, EDM, EDW, EDL, EDD, EDE, EDE, EDM, EDP, EDL, EDF EYD (2x), ENH, EYE, EFE, EFD
entire library	colorless	PTL, PAW, PSL, PFL, PTI, PTW, PIQ, ADL
E-terminal subset of library (EXX)	colorless	EPT (2x), EPP, EPS, EFV (2x), EVA

Figure S2. Sequences identified from library screening. The red beads indicated formation of the oxime product and corresponded to sequences that were highly reactive towards RS-mediated transamination. The colorless beads corresponded to sequences that were not reactive.

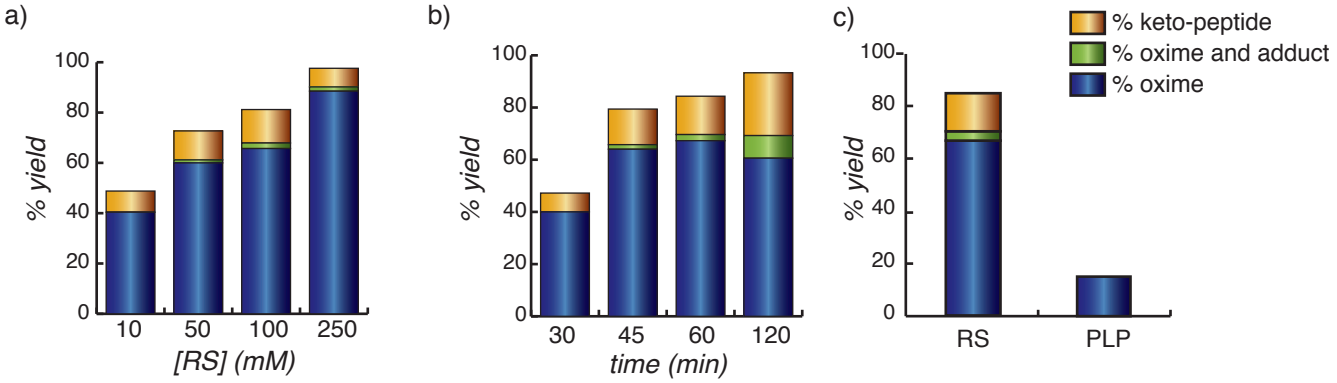


Figure S3. Reaction conditions screen for RS-mediated transamination of the EESWSNAG peptide. (a) The concentration of RS was varied during a 1 h reaction at pH 6.5. Although library screening was done using 10 mM RS for a 1 h reaction, higher yields were found with higher concentrations. (b) Using 100 mM RS at pH 6.5, the reaction time was varied. Although the total conversion was higher, the use of longer reaction times led to increased amounts of adduct. (c) To see if the EE-terminal sequence identified by library screening was an optimal sequence for RS in particular or a sequence that transaminated well with any reagent, conversion using 100 mM PLP for 1 h pH 6.5 was compared to that achieved using 100 mM RS under the same conditions. The yield was significantly higher in the RS-mediated transamination case, indicating that the library screening had indeed identified an optimal transamination reagent/sequence pair.

	% oxime	% oxime and adduct	% transaminated	*% total conversion*
EES	66.1 ± 8.5	1.9 ± 0.6	13.3 ± 3.4	*81.4 ± 5.3*
DES	45.3 ± 13.9	4.2 ± 0.9	3.5 ± 1.4	*53.1 ± 13.6*
AES	45.3 ± 13.9	0 ± 0	4.9 ± 1.4	*46.1 ± 16.7*
PES	0 ± 0	0 ± 0	0 ± 0	*0 ± 0*
EPS	27.1 ± 8.3	0 ± 0	0.9 ± 0.5	*28 ± 8.8*

Figure S4. Peptides of the form XXXWSNAG were synthesized to compare the reactivity of various N-terminal sequences towards RS-mediated transamination. Listed here are the percentage errors after the reaction (standard conditions) was performed in triplicate.

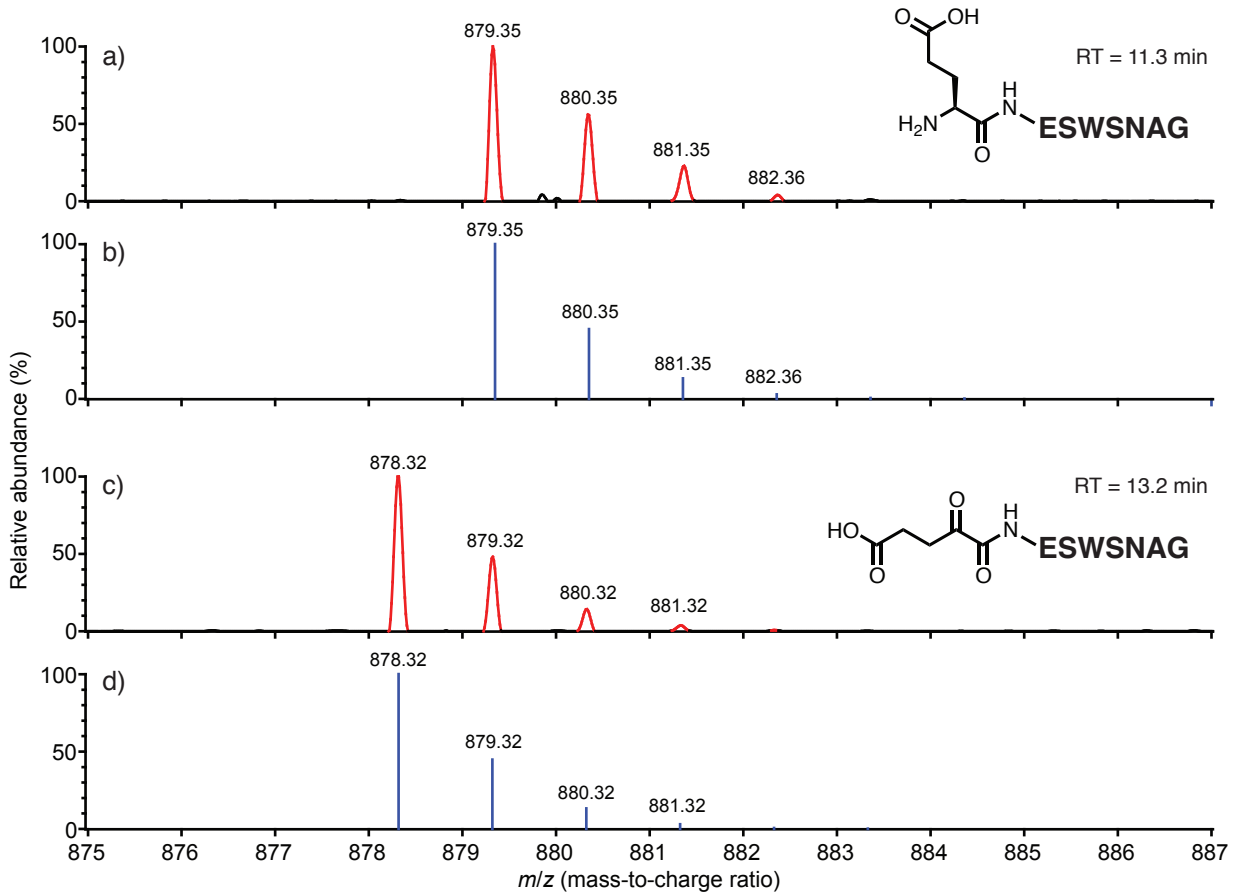


Figure S5. Comparison between measured (red) and theoretical (blue) isotopic distributions of peptide ions measured from the 1 hour reaction of EESWSNAG with 100 mM Rapoport's salt. The spectra correspond to the measured (a) and theoretical (b) isotopic distributions for the $[M + H]^+$ ion of the unmodified peptide ($M = C_{36}H_{50}N_{10}O_{16}$; retention time = 11.3 minutes); and measured (c) and theoretical (d) isotopic distributions for the $[M + H]^+$ ion of the transaminated keto-peptide ($M = C_{36}H_{47}N_9O_{17}$; retention time = 13.2 minutes). Theoretical isotopic distributions were calculated from the natural abundances of the isotopes using MassLynx software (version 4.1, Waters, Milford, MA).

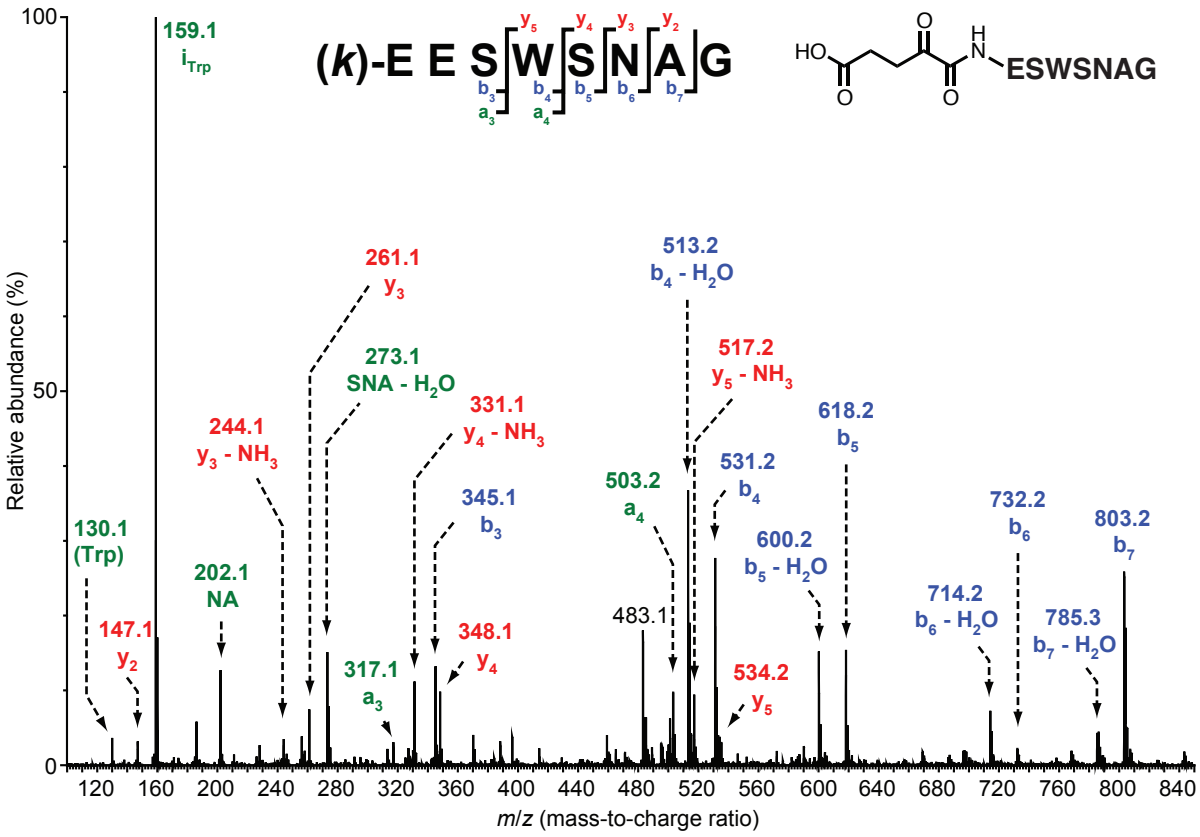


Figure S6. Tandem mass (MS/MS) spectrum and corresponding sequence map resulting from collision-induced dissociation (CID) of the singly charged positive ion at m/z = 878.3, which was due to the $[M + H]^+$ ion of the transaminated form of the peptide EESWSNAG (precursor ion shown in Fig. S5). Immonium internal fragment ions are denoted by "i" and the amino acid code. Internal cleavage fragment ions are labeled with their respective amino acid sequences. The fragment ion at m/z = 130.1 is due to the tryptophan side chain.[4] Fragment ion masses were consistent with N-terminal transamination of the peptide.

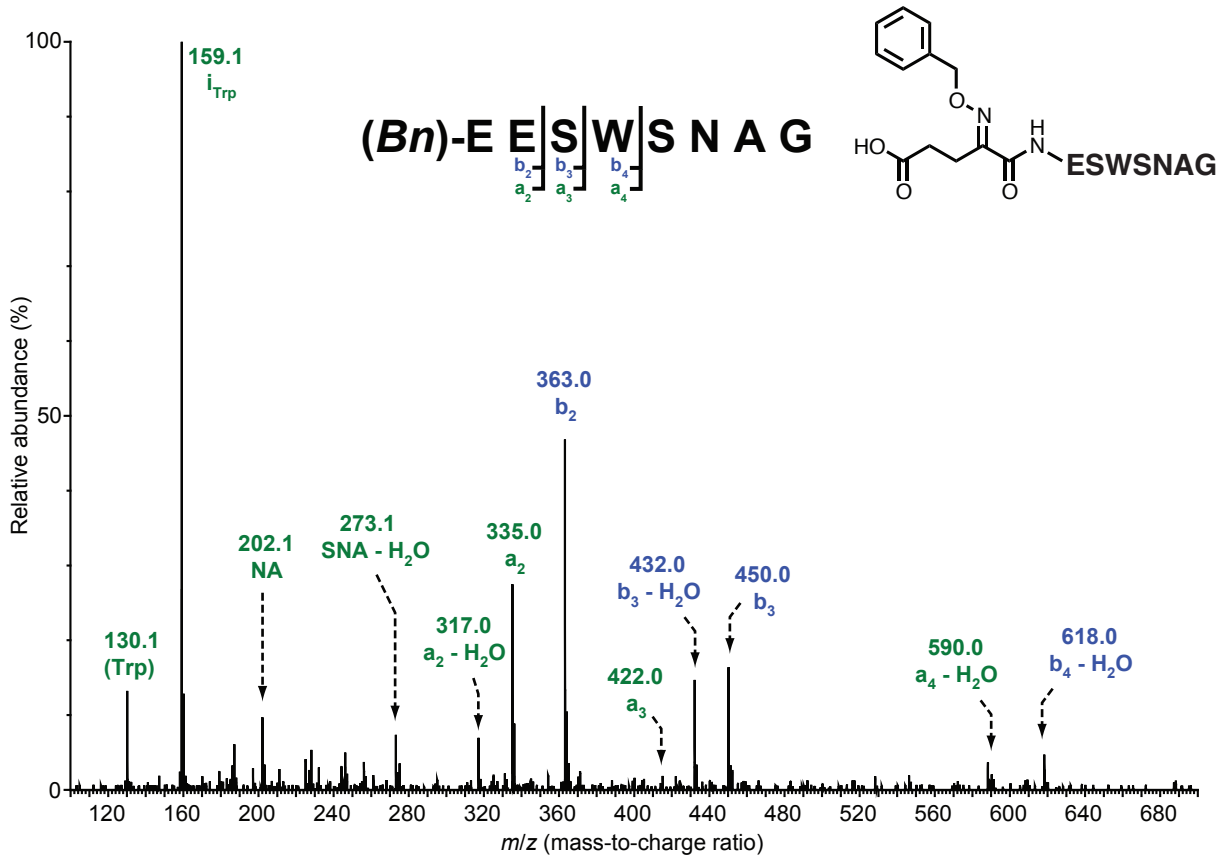


Figure S7. MS/MS spectrum and corresponding sequence map resulting from CID of the singly charged positive ion at m/z =983.5, which was due to the $[M + H]^+$ ion of the benzyloxime modified peptide, (Bn)-EESWSNAG. Fragment ion masses were consistent with the N-terminal oxime.

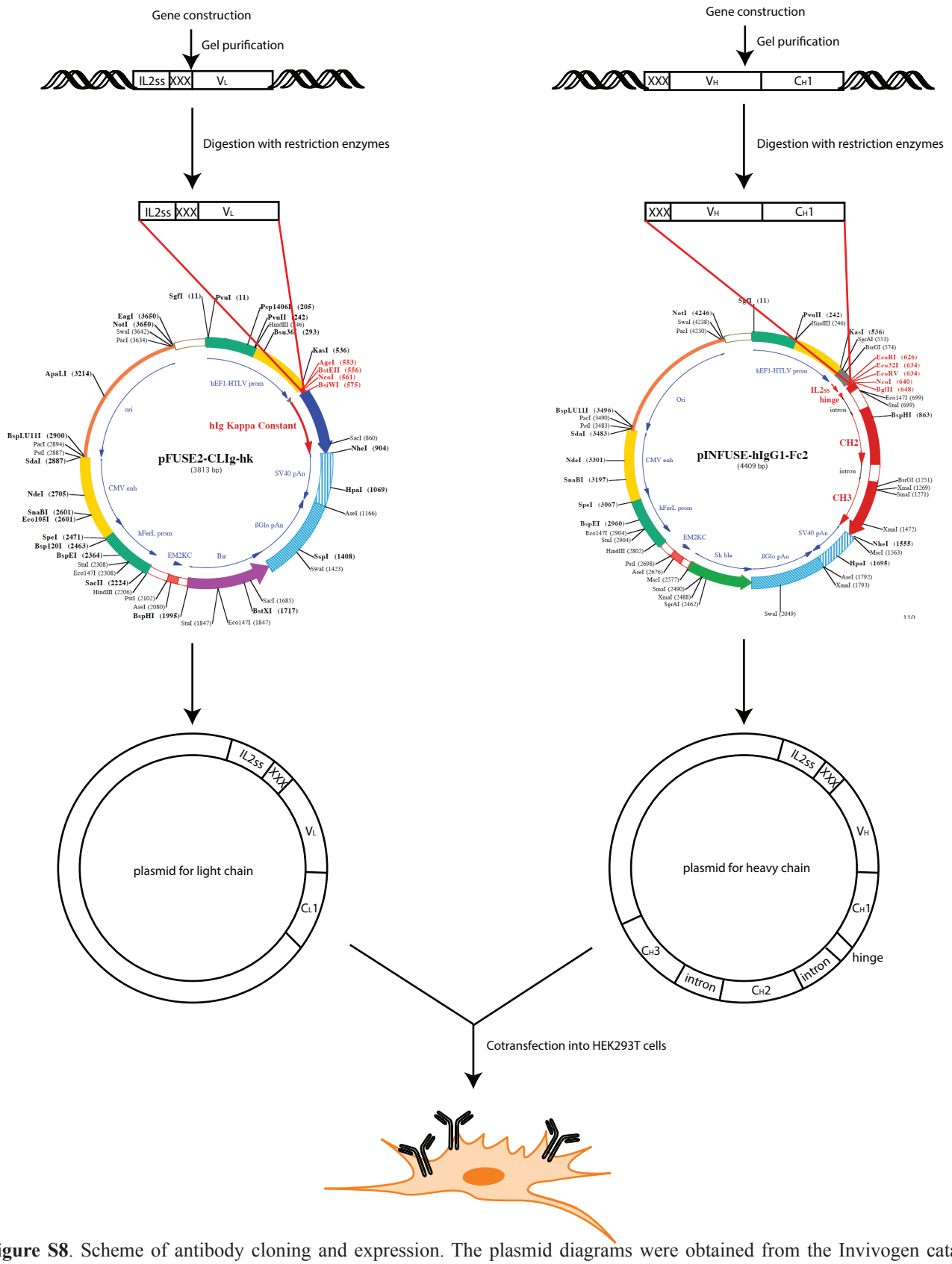


Figure S8. Scheme of antibody cloning and expression. The plasmid diagrams were obtained from the Invivogen catalog. "XXX" represents the three N-terminal residues that were extended in the mutant expressed to include the EES motif.

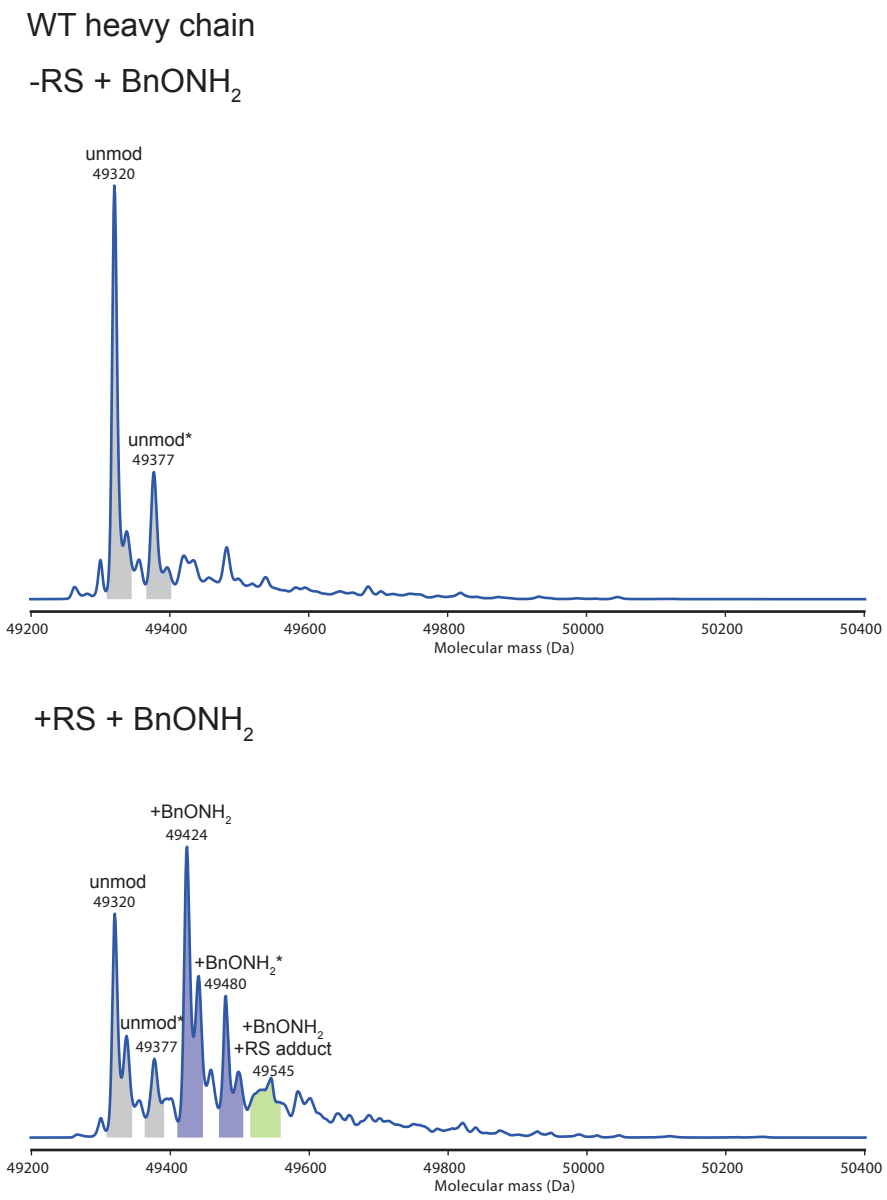


Figure S9. Deconvoluted LCMS spectra of the heavy chain of wild-type anti-HER2 antibodies after transamination using RS, followed by oxime formation with $BnONH_2$. The full-sized antibodies were reduced with DTT, and the reduced cysteines were capped with iodoacetamide before LCMS analysis. The asterisk denotes peaks with an additional iodoacetamide attached to the proteins. The shoulder peaks observed were 18 mass units from the main peak, and likely resulted from the addition of water.

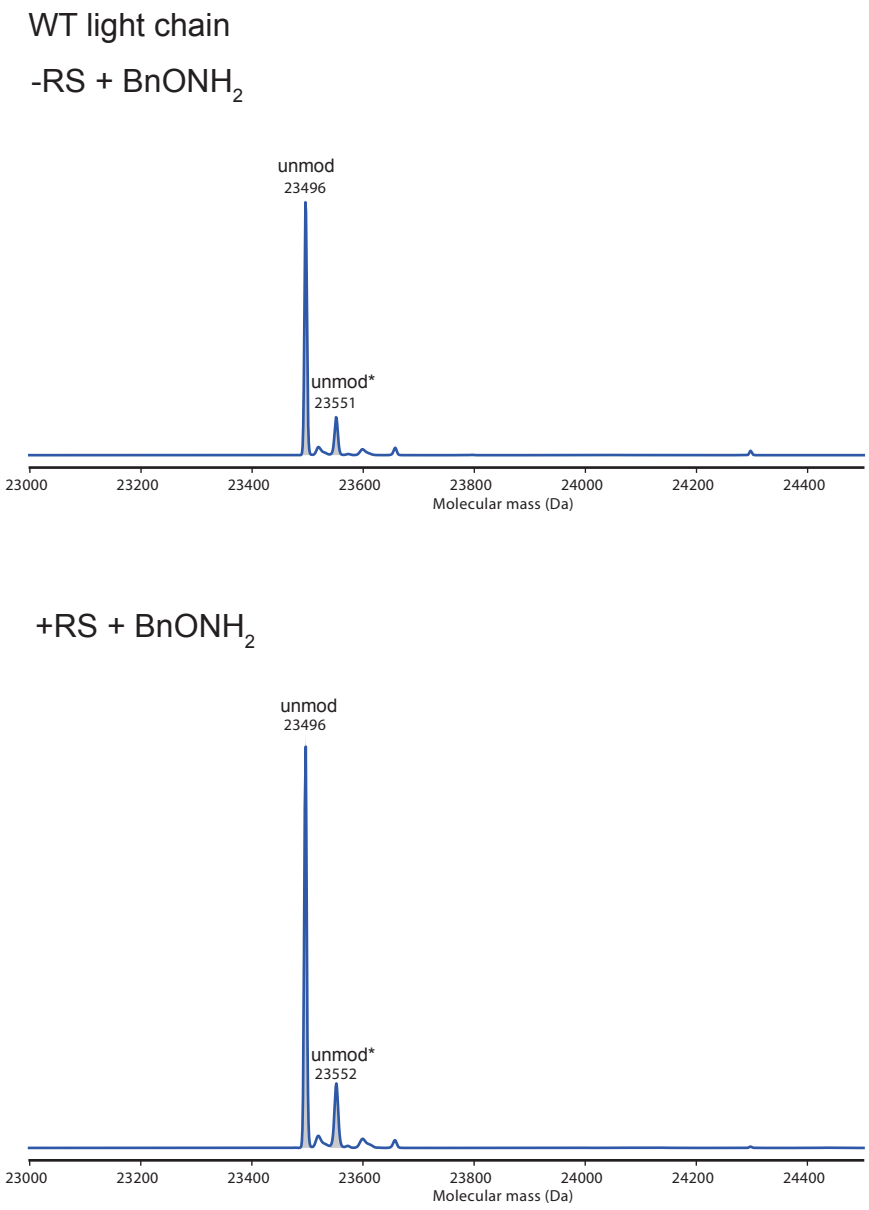


Figure S10. Deconvoluted LCMS spectra of the light chain of wild-type anti-HER2 antibodies after transamination using RS, followed by oxime formation with $BnONH_2$. The full-sized antibodies were reduced with DTT, and the reduced cysteines were capped with iodoacetamide before LCMS analysis. The asterisk denotes peaks with an additional iodoacetamide attached to the proteins.

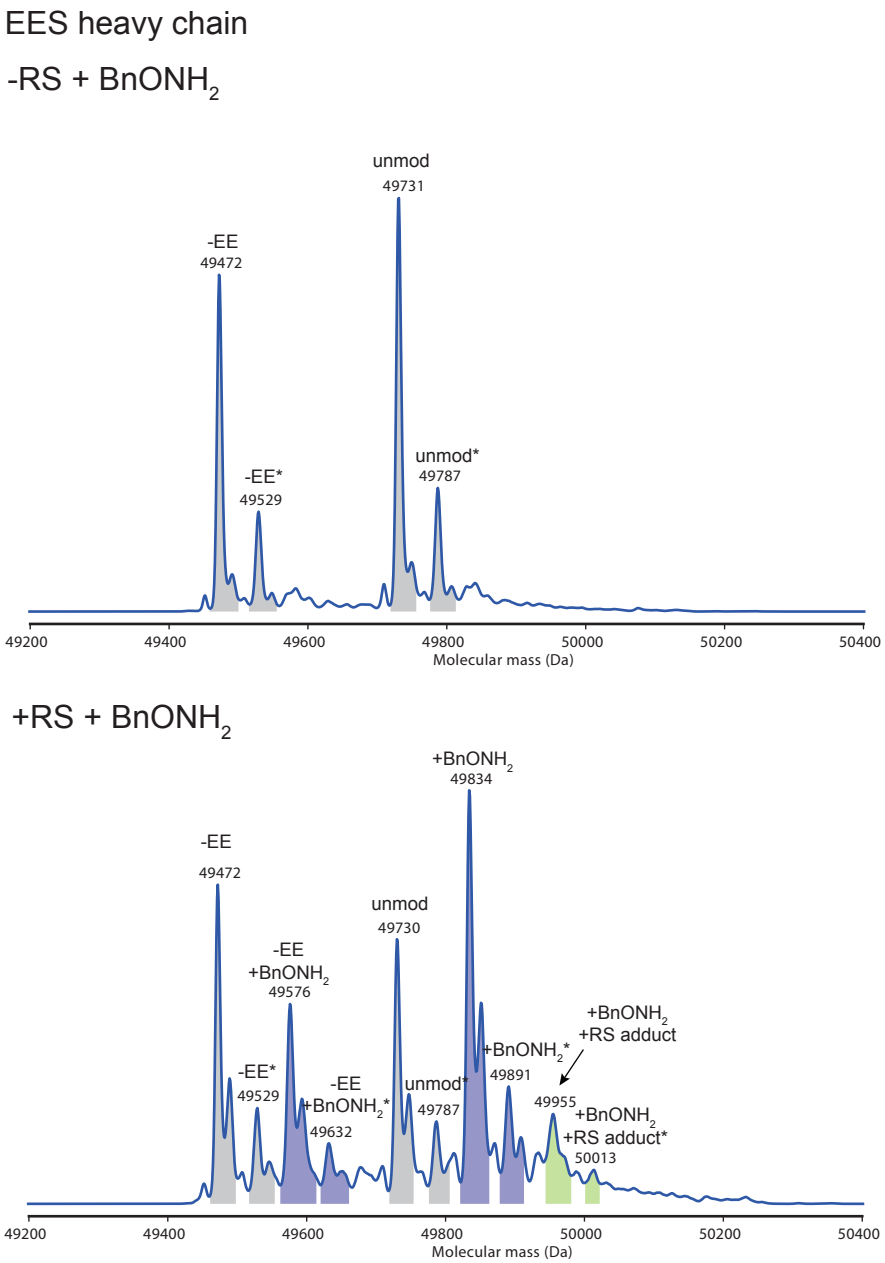


Figure S11. Deconvoluted LCMS spectra of the heavy chain of EES-terminal anti-HER2 antibody mutants after transamination using RS, followed by oxime formation with $BnONH_2$. The full-sized antibodies were reduced with DTT, and the reduced cysteines were capped with iodoacetamide before LCMS analysis. The peak "-EE" refers to light chains which have serine termini due to IL2 signal sequence improper cleavage. The asterisk denotes peaks with an additional iodoacetamide attached to the proteins. The shoulder peaks observed were 18 mass units from the main peak, and likely resulted from the addition of water.

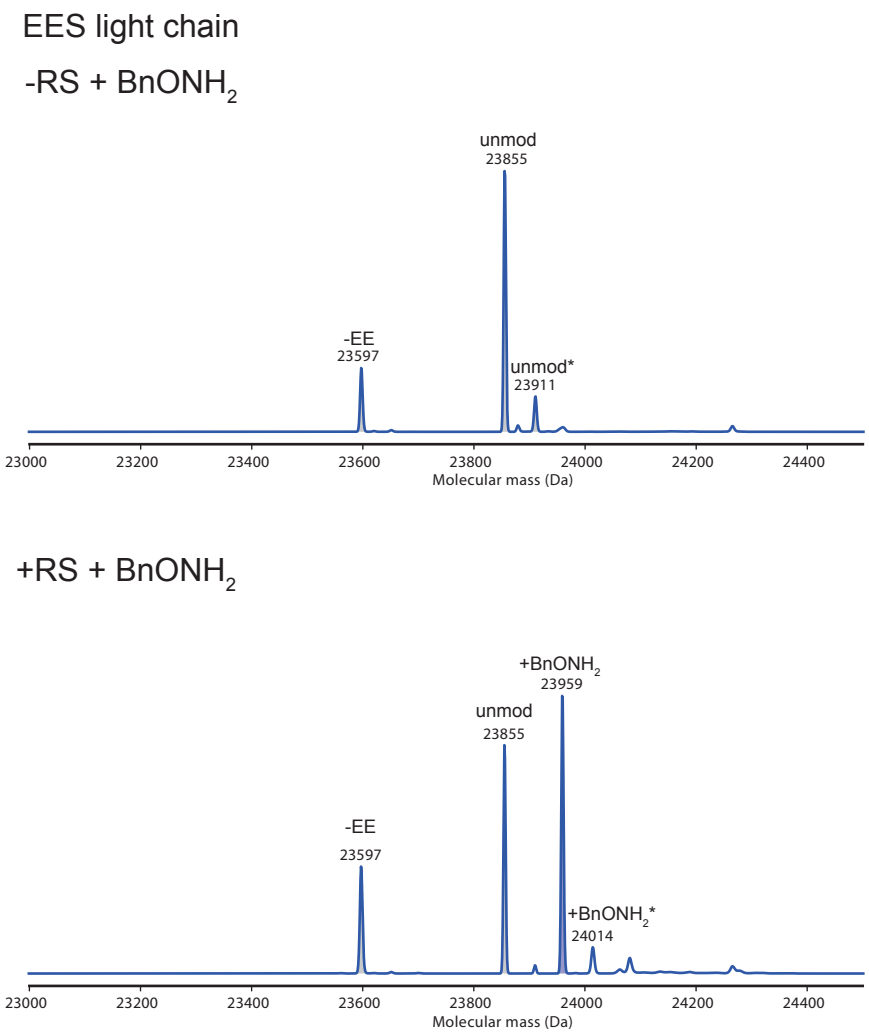
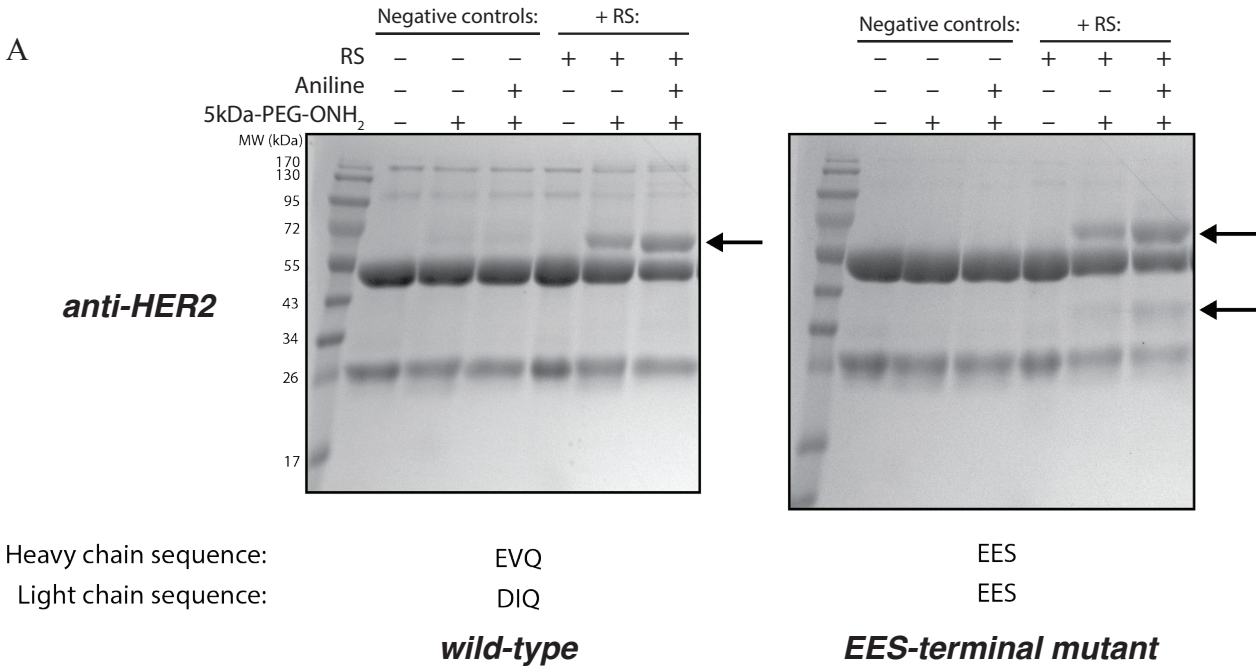


Figure S12. Deconvoluted LCMS spectra of the light chain of EES-terminal anti-HER2 antibody mutants after transamination using RS, followed by oxime formation with $BnONH_2$. The full-sized antibodies were reduced with DTT, and the reduced cysteines were capped with iodoacetamide before LCMS analysis. The peak "-EE" refers to light chains which have serine termini due to IL2 signal sequence improper cleavage. The asterisk denotes peaks with an additional iodoacetamide attached to the proteins.



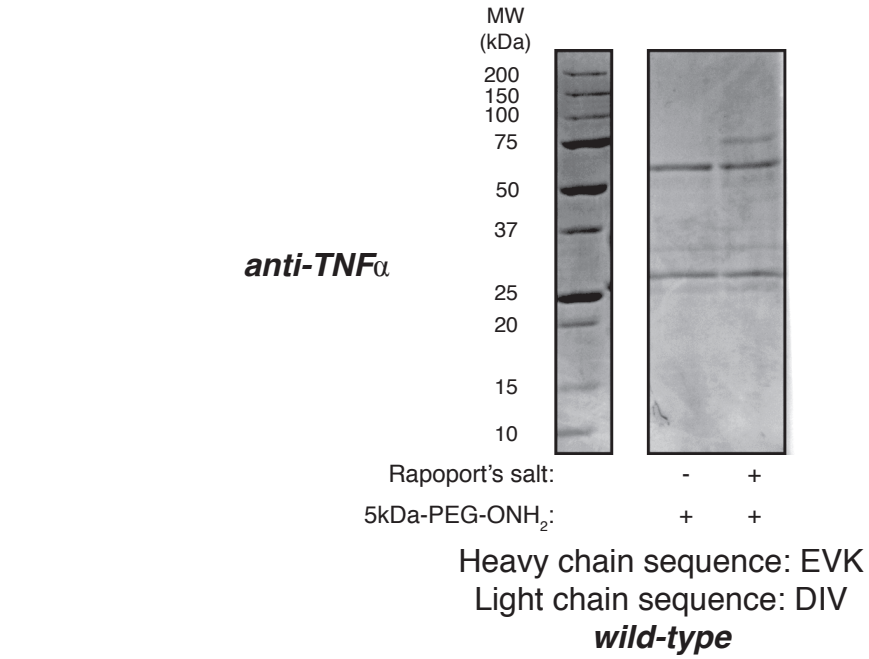


Figure S13. (A) SDS-PAGE gel showing attachment of a 5 kDa poly(ethylene glycol) (PEG) alkoxyamine to the light and heavy chains of the anti-HER2 antibody wild-type and EES-mutant after RS-mediated transamination. The arrows point to the bands corresponding to heavy and light chain proteins with PEG attachment. (B) SDS-PAGE gel showing attachment of a 5 kDa poly(ethylene glycol) (PEG) alkoxyamine to the light and heavy chains of the anti-TNFα antibody.

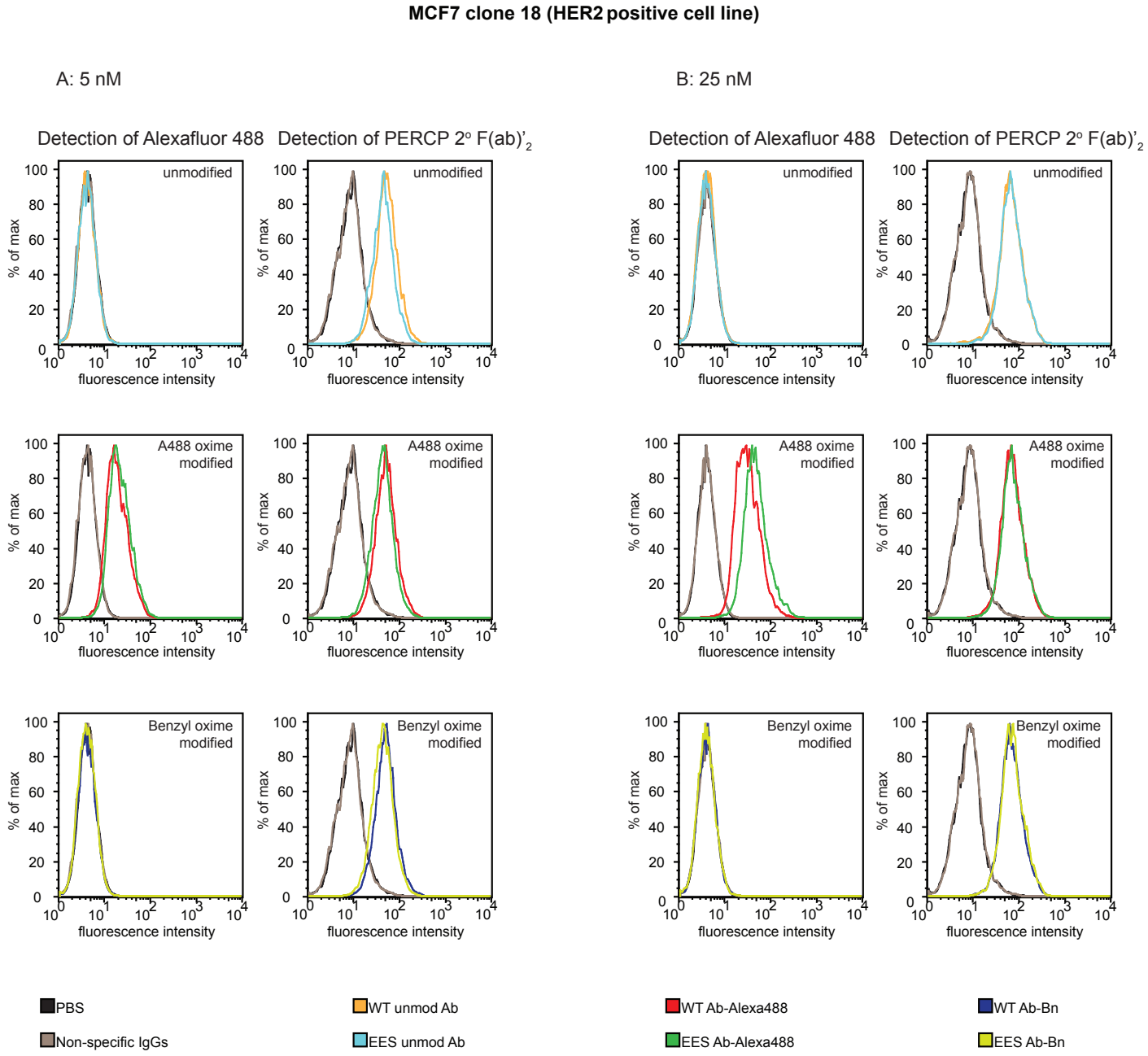


Figure S14. The antigen binding ability of the wild type (WT) and EES-terminal mutants of the anti-HER2 antibody post-modification (transaminated by Rapoport's salt, followed by oxime formation with $BnONH_2$ and AlexaFluor488-ONH_2) was confirmed using flow cytometry. MCF7 clone 18 cells (HER2 positive cell line) were subjected to 5 and 25 nM of wild type and EES antibodies with and without modification. The binding of antibody modified with AlexaFluor488 was detected, while the unmodified or $BnONH_2$ oxime modified ones showed no fluorescent shift (histogram on the left). The bound antibodies were also detected using anti-human IgG Fc PerCP-conjugated 2^o $F(ab')_2$. Both wild type and EES antibodies modified with the $BnONH_2$ and AlexaFluor488-ONH_2 retained similar binding affinity and specificity to the unmodified ones as shown in the right histogram. Non-specific human IgG1 and PBS were used as negative control agents.

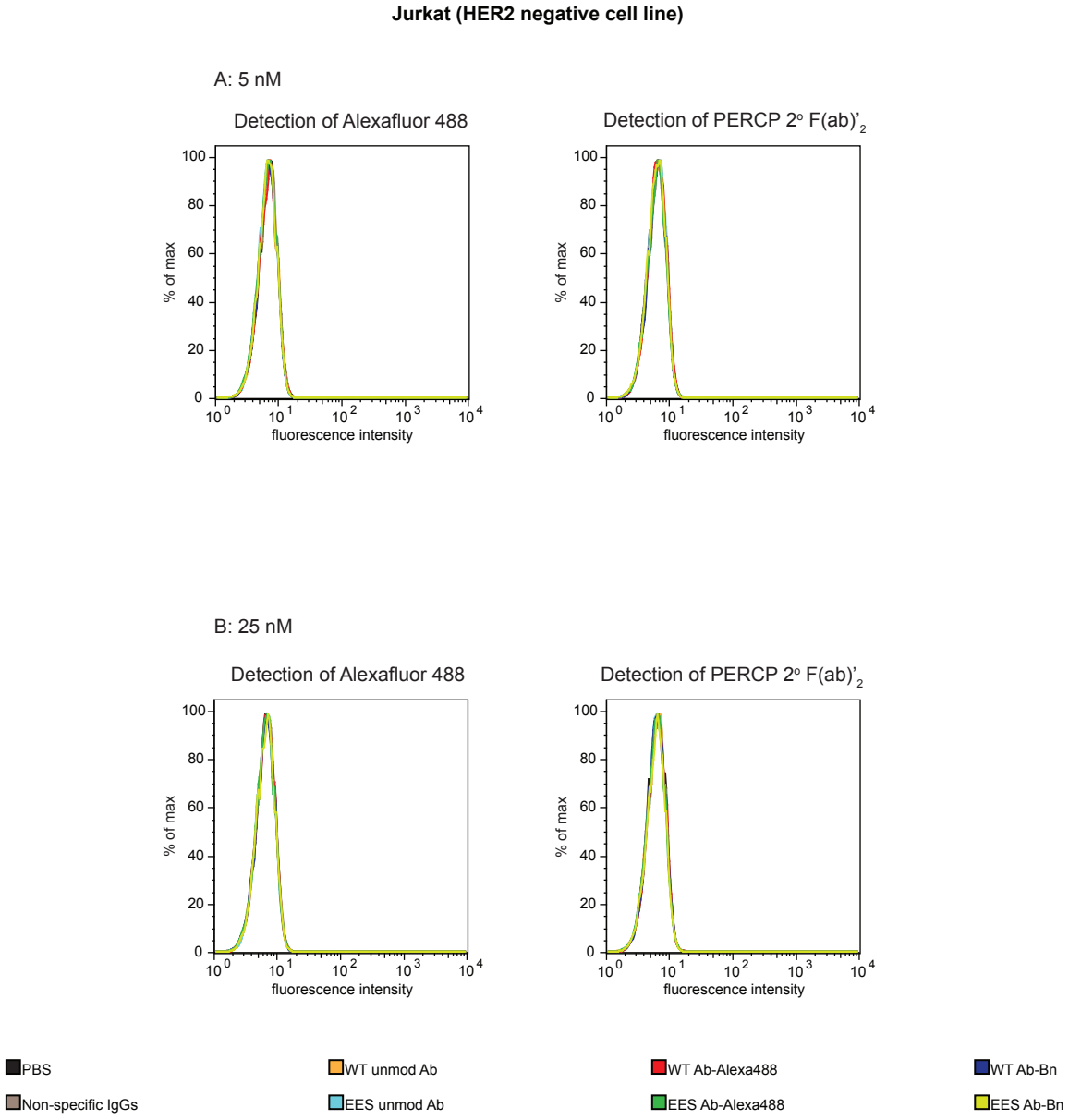


Figure S15. Jurkat cells (HER2 negative cell line) were subjected to 5 and 25 nM of wild type and EES antibodies with and without modification. The negative control cell line did not bind to either unmodified or modified anti-HER2 antibodies.